DEPARTMENT OF AGRICULTURE, CEYLON.

BULLETIN No. 65.

EXPERIMENTS WITH THE GREEN MUSCARDINE FUNGUS ON RHINOCEROS BEETLE LARVÆ.

BY

G. BRYCE, B.Sc.,

Assistant Botanist and Mycologist.

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DEPARTMENT OF AGRICULTURE, CEYLON.

BULLETIN No. 65.

EXPERIMENTS WITH THE GREEN MUSCARDINE FUNGUS ON RHINOCEROS BEETLE LARVÆ.

THE green Muscardine fungus, *Metarrhizium Anisopliæ* (Metsch.) Sor., has received a considerable amount of attention as a parasite on many insects destructive to economic crops and as a possible means of control of such insects. Since 1879, when Metschnikoff first found the fungus as a parasite on cockchafer larvæ on wheat in Russia, repeated attempts have been made to employ the fungus as a means of controlling insect pests in the field. In 1884, Krassilstchik [1] inoculated fields of sugar beet attacked by a curculio, and claimed that 50 per cent. to 80 per cent. of the insects were infected within ten to twelve days, but Pospielov [2] in 1913 stated that Krassilstchik's attempts to disseminate the fungus artificially were unsuccessful. Delacroix found it on silk worms in France in 1893, and in the same year Hart found it in the West Indies on froghoppers in Trinidad sugar cane fields. Rorer [1] in 1910 found that the fungus caused a high mortality among froghoppers in Trinidad sugar cane fields, and devised an apparatus for obtaining large quantities of spores for application on a field scale. Williams,[3] however, reports from Trinidad in 1921 that attempts to disseminate the fungus have as yet given inconclusive results. Speare [4] in 1912 recorded the fungus from Hawaii on sugar cane borer beetle, and from his infection experiments he concluded that the fungus is "less virile than has been reported in many cases." The fungus is already present in the fields and helps to hold the insects in check, but such low mortalities were obtained in the laboratory under supposedly ideal infection conditions, that it cannot be accepted as a "cure-all." Friederichs [5] in 1914 stated that infection experiments in Samoa with the fungus on Rhinoceros beetle had not been successful.

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The fungus has been reported from several other countries and on many other insects, and has therefore a wide geographical distribution, and is apparently practically indifferent as to host insects.

The fungus was brought to Peradeniya by Friederichs in 1914, who brought material collected by him in various places. The fungus had not been recorded in Ceylon up to that time, but in 1916 it was obtained by Petch at Hakgala on a cricket, and it has lately been found by Hutson on Rhinoceros beetle (*Oryctes rhinoceros*) larvæ under observation in the Entomological Laboratory at Peradeniya. The Hakgala fungus should be considered as indigenous, as it is extremely improbable that it is an escape from Friederichs' imported fungi.

Doane [6] records that the Rhinoceros beetle was first noted in Samoa in 1910, and it is supposed that it was introduced from Ceylon in soil and vegetable refuse used as packing for rubber stumps. Friederichs at that time was plant pathologist in Samoa, and in the course of his investigations he found larvæ which were parasitized by this fungus. In 1914 he visited Peradeniya, and cultures of the fungus were made from the material in his possession, namely, (1) larva found in the Philippines, (2) larva found in Samoa, (3) larva found in Malaya, and (4) a pure culture of the fungus from Hawaii (named P. H. hereafter).

The Fungus in Artificial Culture.

Cultures on rice agar were made by Petch on April 27 from Friederichs' material, but were accidentally destroyed on May 8, before the fungus had made any apparent growth. Tube cultures of the same date kept till May 28 did not show any sign of *Metarrhizium*.

At the same time larvæ inoculated from the Philippine and Malayan material about the end of April, and kept until the end of May in closed glass jars containing a mixture of soil and wood shavings, were then found to be dead and covered with a good growth of *Metarrhizium*. Larvæ inoculated from the Hawaiian and Samoan material gave no growth of the fungus, and subsequent attempts to establish pure cultures were equally unsuccessful in these two cases.

From the above larvæ infected with the Philippine and Malayan strains pure cultures of the fungus were established. The fungus made good growth and produced spores abundantly on both the media employed, namely, boiled rice and a rice bouillon-cane sugar agar. These two strains were kept in pure culture for four months while the infection experiments were in progress. The spores used in the infection experiments were taken from fresh cultures.

The spores germinate readily in distilled water ; in hanging drop cultures the Philippine strain was found to make much more rapid and abundant growth than the Malayan strain.

Infection Experiments.

Rorer [1] records that 5 days after inoculation of 50 adult froghoppers inoculated by spraying with water containing spores, all were dead, and 19 showed a good covering of the fungus.

Speare [4] found that many sugar cane borer beetles died off and showed the fungus 14 days after inoculation, the highest mortality being obtained when the spores were applied directly to the body of the insect.

In these two cases, therefore, the fungus rapidly caused death among the insects, and the effect of captivity on the health of the insect could be to a great extent ignored.

The following are the results of infection experiments with *Metarrhizium* on Rhinoceros beetle larvæ:—

Philippine Strain.

Experiment 1.—This was a preliminary experiment to obtain quantities of spores to establish pure cultures. A number of larvæ were inoculated and placed in glass dishes containing a mixture of soil and wood shavings, and covered by close-fitting lids. Inoculations were made by smearing an ample quantity of spores on the dorsal surface of the larva. The consistency of the soil and wood-shavings mixture was heavy and wet, and provided obviously unhealthy conditions for the larvæ. At end of a month all the larvæ present were dead, and were covered with a copious growth of *Metarrhizium*.

Experiment 2.—The living conditions in the last experiment being unfavourable to the larvæ, a change was made and inoculated larvæ were placed in kerosene tins, open at one end, and containing coconut palm refuse, such as fibrous husks of the nuts, leaf bases, &c.

June 13.—Inoculated 7 larvæ.
July 13.—Living larvæ 6, dead 1 ; no fungus present.
July 15.—Reinoculated 4 larvæ, 2 larvæ lost.
July 22.—Living larvæ 3, living pupa 1, dead larva 1 ; no fungus present.
July 23.—Reinoculated 3 living larvæ.
July 31.—Living larvæ 2, living pupa 1.
August 6.—Living larva 1, living pupæ 2.
September 2.—Dead, 1 larva and 2 pupæ ; fungus present on all.

In this experiment therefore the larvæ were in captivity for 54 days ; they had been inoculated with the fungus on three occasions, and were still living. The fungus became apparent on the dead larvæ at about 81 days from the first inoculation.

Experiment 3.—A second test of the fungus on the larvæ under unhealthy conditions was made. In this case the closed glass jars were again employed, but coconut palm refuse was used to fill them.

July 25.—Inoculated 8 larvæ.
July 31.—Living larva 1, dead 7 ; no fungus evident.
August 6.—Living larva 1 ; no fungus on remainder.
September 2.—Living larva 1 ; no fungus on remainder.

In this case the infected larva lived under unhealthy conditions in a closed, moisture-laden chamber for 39 days, at the end of which time none of the other inoculated larvæ showed any sign of the fungus. The mortality may therefore have been due to other causes.

Experiment 4.—Larvæ were collected and classified into big and small, to try the effect of the fungus on them at different stages of development. After inoculation the larvæ were placed in open kerosene tins containing coconut palm refuse.

Small Larvæ.

July 25.—Inoculated 12 small larvæ.
July 31.—Living larva 1, dead 11 ; no fungus present.
August 6.—Living larva 1 ; remainder show no fungus.
September 2.—Living larva 1 ; remains of dead larvæ disappeared.

The small larvæ were evidently unable to withstand the change of living conditions under captivity, and died off rapidly. Death did not appear in any case to be due to the fungus.

Big Larvæ.

July 25.—Inoculated 8 big larvæ.
August 6.—Living 7, dead 1 ; no fungus present.
August 8.—Reinoculated 6 larvæ and transferred to closed glass dish.
August 11.—Living 5, dead 1 ; no fungus present.
September 2.—Dead 6 ; fungus present on all.

Of the 7 larvæ inoculated (deducting the one which disappeared) 6 died and developed the fungus 39 days from the

first inoculation and 25 days after reinoculation. It is to be presumed that here, again, the long captivity must have had an injurious effect on the larvæ.

Experiment 5.—Open kerosene tins containing coconut palm refuse were employed. Portions of rice cultures of the fungus were placed in contact with each larva in the kerosine tin.

October 20.—Inoculated 4 larvæ in one tin and 5 larvæ in another.
December 5.—Larvæ all dead and fungus present on all.

Here the larvæ were in captivity for 46 days from the time of inoculation to copious development of the fungus.

Malayan Strain.

Experiment 6.—This is a repetition of experiment 3 with the Malayan strain.

Small Larvæ.

July 25.—Inoculated 12 small larvæ.
July 31.—Living 3, dead 9 ; no fungus present.
August 6.—Living 2, dead 10 ; no fungus present.
September 2.—Living 1, dead 11 ; no fungus present.

In this case the young larvæ died off less rapidly, but no fungus developed on any of the dead larvæ. Death may thus have been due to other causes.

Big Larvæ.

July 25.—Inoculated 8 big larvæ.
July 31.—Living 7, dead 1 ; no fungus present.
August 6.—Living 7 ; no fungus present on the dead larva.
August 8.—Reinoculated 6 larvæ, and transferred to closed glass dish.
August 11.—Living 6.
September 2.—Dead 6 ; fungus present on all.

Experiment 7.—A repetition of experiment 4 with the Malayan strain. Larvæ placed in open kerosene tins containing coconut palm refuse. Portions of rice cultures of the fungus were placed in contact with each larva in the kerosene tin.

October 20.—Inoculated 4 larvæ in one tin and 5 in another.
December 5.—Larvæ all dead and fungus present on all.

Conclusions.

The green Muscardine fungus is apparently not strongly pathogenic to Rhinoceros beetle larvæ. This corresponds with Speare's observations on the effect of the fungus on sugar cane borer beetle. From the time of inoculation of the Rhinoceros beetle larvæ to the development of the fungus on the larvæ, periods varying from 39 days to 81 days elapsed. Larvæ which had been inoculated on three occasions were in one case still living 54 days after the first inoculation. The fungus will tend to check the increase of the beetle under natural conditions, but judging from its feeble parasitism on the larvæ it is improbable that natural infection could be increased by artificial field infections.

The evidence from each experiment is to the effect that the fungus only attacks the larvæ after they have been in captivity for a considerable period, and have accordingly suffered to some extent a reduction in vitality. Healthy larvæ living under normal conditions are apparently not very susceptible to the disease. As the fungus is parasitic on many different insects, has a wide geographical range, and is indigenous in Ceylon, it may be concluded that Rhinoceros beetle larvæ in Ceylon are generally subject to infection at some period of their existence. Whether the insects become diseased or not appears to depend on the conditions under which they are living, and epidemics of the disease would occur when conditions favour the fungus and are unfavourable to the insect. Any attempt therefore to increase the incidence of the disease by artificial infection is wholly controlled by the conditions obtaining at the time. If conditions are unfavourable it is useless to make the attempt, while if they are favourable the fungus will spread and the disease become epidemic without artificial aid. In the case of another insect pest and fungus disease investigated, the authors [7] point out that "advocating artificial infection or encouraging it does not serve the best interests of the farmer, since his attention is thus diverted from other and more efficient methods of combating the pest." The same remark applies with even greater force in the present instance.

Artificial infection with the green Muscardine fungus as a means of controlling the Rhinoceros beetle on coconuts is therefore not advised.

G. BRYCE.

September, 1922.

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